Quantitative Transcriptomics using Designed Primer-based Amplification.

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Public Summary:
A new method was developed to amplify limiting amounts of mRNA for sequencing, enabling sequencing technology to be quantitative for very low levels of starting material. The method was evaluated in a pluripotent stem cell model, revealing that many genes previously not characterized as mesodermal and endodermal are expressed as these cell types emerge.

Scientific Abstract:
We developed a novel Designed Primer-based RNA-sequencing strategy (DP-seq) that uses a defined set of heptamer primers to amplify the majority of expressed transcripts from limiting amounts of mRNA, while preserving their relative abundance. Our strategy reproducibly yielded high levels of amplification from as low as 50 picograms of mRNA while offering a dynamic range of over five orders of magnitude in RNA concentrations. We also demonstrated the potential of DP-seq to selectively suppress the amplification of the highly expressing ribosomal transcripts by more than 70% in our sequencing library. Using lineage segregation in embryonic stem cell cultures as a model of early mammalian embryogenesis, DP-seq revealed novel sets of low abundant transcripts, some corresponding to the identity of cellular progeny before they arise, reflecting the specification of cell fate prior to actual germ layer segregation.